

Cell adhesion assay

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 An abbreviated version of this protocol was published in eLIFE in Jan 2022

RNA *N*⁶-methyladenosine modulates endothelial atherogenic responses to disturbed flow in mice

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Detailed protocol

This protocol enables the detection of cell adhesion assay with THP-1 adhesion to HUVECs in response to oscillatory shear stress (OSS)

1. Prepared THP-1 cells and cultured with 1640 medium containing 10% FBS.
2. Prepared HUVECs (P5-P6) on the plate and in response to OSS.
3. THP-1 cells were collected into a 15ml centrifuge tube at 1000rpm for 5min. Discarded the culture medium and resuspended with PBS buffer, repeated 2-3 times.
4. THP-1 cells were labeled with CellTrace calcein red-orange AM (Thermo Fisher Scientific, catalog C34851); Another dye BCECF-AM (MCE, HY-101883) will also work, and the dye was added to a 15ml centrifuge tube at a ratio of 1:500 with PBS, and incubated at 37 ° C for 30min in darkness.
5. The supernatant was discarded by centrifugation at 1000rpm for 5min, washed three times with PBS, then resuspended with M199 medium, and added to HUVECs according to 2×10⁶ cells per glass plate, and incubated at 37°C for 1 hour.
6. Nonadherent cells were removed by washing 3 times gently with PBS. The numbers of stained adhering cells in 5 random fields were counted for each group under a fluorescence microscope.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Li, B. , Yang, Y. , Jiang, H. and Liang, D. (2022). Cell adhesion assay. Bio-protocol Preprint. bio-protocol.org/prep1956.
2. Li, B., Zhang, T., Liu, M., Cui, Z., Zhang, Y., Liu, M., Liu, Y., Sun, Y., Li, M., Tian, Y., Yang, Y., Jiang, H. and Liang, D.(2022). RNA *N*⁶-methyladenosine modulates endothelial atherogenic responses to disturbed flow in mice. eLIFE. DOI: [10.7554/eLife.69906](https://doi.org/10.7554/eLife.69906)

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